Laboratory Procedure Manual

Analyte: Chlamydia trachomatis and Neisseria gonorrhoeae

Matrix: **Urine**

Method: C. trachomatis and N. gonorrhea Assay on BDProbeTec Analyzer

Method No.:

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Revised:

as performed by: *Division of AIDS, STD, and TB Laboratory Research*
National Centers for Infectious Diseases
National Centers for Disease Control and Prevention

Contact:

Important Information for Users

The Division of AIDS, STD, and TB Laboratory Research periodically refines these laboratory methods. It is the responsibility of the user to contact the person listed on the title page of each write-up before using the analytical method to find out whether any changes have been made and what revisions, if any, have been incorporated.

Public Release Data Set Information

This document details the Lab Protocol for testing the items listed in the following table:.

Data File Name	Variable Name	SAS Label
Chlmda_d	URXUCL	Chlamydia, urine
	URXUGC	Gonorrhea, urine

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL SIGNIFICANCE

The BDProbeTec CT *Chlamydia trachomatis* and *Neisseria gonorrhoeae* Amplified DNA Assays are based on the simultaneous amplification and detection of target DNA using amplification primers and a fluorescent labeled detector probe. The Strand Displacement Amplification (SDA) reagents are dried in two separate disposable microwell strips. The processed sample is added to the Priming Microwell which contains the amplification primers, fluorescent labeled detector probe, and other reagents necessary for amplification. After incubation, the reaction mixture is transferred to the Amplification Microwell, which contains two enzymes (a DNA polymerase and a restriction endonuclease) necessary for SDA. The Amplification Microwells are sealed to prevent contamination and then incubated in a thermally controlled fluorescent reader which monitors each reaction for the generation of amplified products. The presence or absence of *C. trachomatis* and *N. gonorrhoeae* is determined by relating the BDProbeTec ET MOTA (Method Other Than Acceleration) scores for the sample to pre-determined cutoff values. The MOTA score is a metric used to assess the magnitude of signal generated as a result of the reaction.

Chlamydia trachomatis and *Neisseria gonorrhoeae* infections are the most common sexually transmitted bacterial diseases in the United States. Approximately 4 million new chlamydia cases are estimated to occur each year in the United States with worldwide estimates of approximately 50 million new cases annually (1-3). The incidence of chlamydial infections in women in the US in 1996 was 186.6 per 100,000. The total number of chlamydial infections and gonorrhea cases reported in the US in 1996 were 490,080 and 325,883, respectively (2).

Chlamydia is gram-negative, obligate intracellular bacteria. They form characteristic intracellular inclusions which can be observed in cell culture by light microscopy after special staining is applied (4). *Chlamydia trachomatis* causes cervicitis, urethritis, salpingitis, proctitis and endometritis in women and urethritis, epididymitis and proctitis in men. Acute infections are reported more frequently in men because women often have no symptoms of infection. It has been estimated that 70 - 80% of women and up to 50% of men who are infected experience no symptoms. Many chlamydial infections in women remain untreated which may result in low-grade inflammation in the Fallopian tubes, a leading contributor to infertility. This organism can also be transmitted in the birth canal, potentially resulting in infant conjunctivitis and/or chlamydial pneumonia in newborns (4, 5).

Neisseria gonorrhoeae are gram-negative, oxidase positive diplococci which can be observed in Gram-stained smears of urethral discharges, usually within neutrophils. Culture of *N. gonorrhoeae* can be difficult because the organism does not survive long outside its host and is highly susceptible to adverse environmental conditions such as drying and *extreme* temperatures (6). *Neisseria gonorrhoeae* causes acute urethritis in males, which if untreated can develop into epididymitis, prostatitis, and urethral stricture. In females, the primary site of infection is the endocervix. An important complication in females is development of pelvic inflammatory disease which contributes to infertility (7). Asymptomatic infections occur often in females but infrequently in males.

The current methods for detection of C. *trachomatis* and/or *N. gonorrhoeae* include culture, immunoassays, non-amplified probes, and amplified probes (4, 6, and 7). The development of amplified methods has demonstrated two advantages over non-amplified methods: increased sensitivity, and applicability to a variety of sample types. Historically,

culture has been the "gold standard" for detection of C. *trachomatis.* However, the culture yield varies widely among laboratories, and culture in routine practice is less sensitive than amplified methods. Combining results from multiple methods of *C. trachomatis* detection improves accuracy for evaluating new tests in that infected and uninfected patients can be more reliably identified. For identification of *N. gonorrhoeae*, optimized culture methods continue to be the standard for diagnosing patients with gonococcal infections.

2. SAFETY PRECAUTIONS

a. This reagent pack is for testing endocervical and male urethral swabs and male and female urine specimens with the BDProbeTec ET System.

b. For collection of endocervical swab specimens, use only the BDProbeTec ET *Chlamydia trachomatis I Neisseria gonorrhoeae (CT/GC)* Amplified DNA Assay Endocervical Specimen Collection and DRY TRANSPORT Kit or the BDProbeTec ET *ChlamydIa trachomatis I Neisseria gonorrhoeae (CT/GC)* Amplified DNA Assay Collection Kit for Endocervical Specimens.

c. For collection of male urethral swab specimens, use only the BDProbeTec ET *Chlamydia trachomatis I Neisseria gonorrhoeae (CT/GC)* Amplified DNA Assay Male Urethral Specimen Collection and DRY TRANSPORT Kit or the BDProbeTec ET *Chlamydia trachomatis I Neisseria gonorrhoeae (CT/GC)* Amplified Assay Collection Kit for Male Urethral Specimens.

d. Do not test the *CT/GC* Diluent tube from the BDProbeTec ET *CT/GC* Amplified Assay Collection Kits if received in the laboratory without the swab present. A false negative test result may occur.

e. Use only the BDProbeTec ET Urine Processing Kit for processing urine specimens.

f. Use only the BDProbeTec ET Pipettor and BDProbeTec ET Pipette tips for the transfer of processed samples to Priming Microwells and the transfer of samples from the Priming Microwells to the Amplification Microwells.

g. Do not Interchange or mix kit reagents from kits with different lot numbers

h. Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimen. "Standard Precautions" 1114 and institutional guidelines should be followed in handling all Items contaminated with blood and other body fluids.

i. Use established laboratory practices when disposing of used pipette tips, sample tubes, Priming Microwells and other disposables. Discard disposables carefully. Seal and dispose of waste containers when they are *3/4* full or daily (whichever comes first).

j. The BDProbeTec ET Diluent *(CT/GC)* and *CT/GC* Diluent tube contain dimethyl sulfoxide (DMSO). DMSO is harmful, with possible risk of irreversible effects through inhalation, contact with skin or If swallowed. Avoid contact with eyes. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water.

k. Reagent pouches containing unused Priming Microwells and Amplification Microwells MUST are carefully resealed after opening. Verify that a desiccant is present prior to resealing the reagent pouches.

l. The plate containing the Amplification Microwells properly sealed with the Amplification Sealer prior to moving the plate from the BDProbeTec ET Priming and Warming Heater to the BDProbeTec ET Instrument. Sealing ensures a closed reaction for amplification and detection and is necessary to avoid contamination of the instrument and work area with amplification products. Do not remove sealing material from microwells at any time.

m. Priming Microwells with residual fluid (after transfer of fluid from the Priming Microwells to the Amplification Microwells) represent a source of target contamination. Carefully seal Priming Microwells with plate sealer prior to disposal.

n. To prevent contamination of the work environment with amplification products, use the disposal bags provided in the Reagent Packs to dispose of tested Amplification Microwells. Make sure the bags are properly closed before disposal.

o. Although dedicated work areas are not required because the BDProbeTec ET design reduces the possibility of amplicon contamination in the testing environment, other precautions for controlling contamination, particularly to avoid contamination of specimens during processing, are necessary

p. Because of the potential for false positivity with some non-gonococcal *Neisseria* found in the respiratory tract, contamination of reagents and specimens with respiratory aerosols should be avoided.

q. CHANGE GLOVES after removing and discarding caps from lysed samples and controls to avoid cross-contamination of specimens. If gloves come in contact with specimen or appear to be wet, immediately change gloves to avoid contaminating other specimens. Change gloves before leaving work area and upon entry into work area.

r. In the event of contamination of the work area or equipment with samples or controls, thoroughly clean the contaminated area with 1 % (v/v) sodium hypochlorite with Alconox and rinse thoroughly with water. Allow surface to dry completely before proceeding. In case of a spill on the Lysing Rack: The rack may be immersed in 1 % sodium hypochlorite with Alconox for 1 - 2 min. Do not exceed 2 minutes. Thoroughly rinse the rack with water and allow to air dry.

s. Clean the entire work area - counter tops and instrument surfaces - with 1 % (vlv) sodium hypochlorite with Alconox on a daily basis. Thoroughly rinse with water. Allow surfaces to dry completely before proceeding with additional testing.

t. Contact Technical Services in the event of an unusual Situation, such as a spill into the BDProbeTec ET instrument or DNA contamination that cannot be removed by cleaning.

3. COMPUTERIZATION; DATA SYSTEM MANAGEMENT

a. Specimens received from various research studies labeled by the specimen ID, collection date, and type of sample (i.e. urine). Specimens tested in this laboratory with this procedure are derived from participants consented and enrolled in CDC IRB approved investigational studies.

b. After the data is calculated and the final values are approved by the reviewing supervisor for release, all results are entered onto the specific study data file.

4. SPECIMEN COLLECTION, STORAGE, AND HANDLING PROCEDURES; CRITERIA FOR SPECIMEN REJECTION

The BDProbeTec ET System is designed to detect the presence of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in endocervical swabs, male urethral swabs and male and female urine specimens using the appropriate collection method.

The only devices that can be used to collect swab specimens for testing on the BDProbeTec ET Instrument are:

BDProbeTec ET *Chlamydia trachomatis I Neisseria gonorrhoeae (CT/GC)* Amplified DNA Assay Endocervical Specimen Collection and DRY TRANSPORT Kit

BDProbeTec ET *Chlamydia trachomatis I Neisseria gonorrhoeae (CT/GC)* Amplified DNA Assay Male Urethral Specimen Collection and DRY TRANSPORT Kit

BDProbeTec ET *Chlamydia trachomatis I Neisseria gonorrhoeae (CT/GC)* Amplified DNA Assay Collection Kit for Endocervical Specimens

BDProbeTec ET *Chlamydia trachomatis I Neisseria gonorrhoeae (CT/GC)* Amplified Assay Collection Kit for Male Urethral Specimens

Urine specimens must be collected in a sterile, plastic, preservative-free, specimen collection cup. Use only the BDProbeTec ET Urine Processing Kit for processing urine specimens.

a. After collection, the endocervical swabs and the male urethral swabs must be stored and transported to the laboratory and/or test site at 2 - 27°C within 4-6 days. NOTE: If specimens cannot be transported directly to the testing laboratory under ambient temperatures (15 - 27°C) and must be shipped, an insulated container with ice should be used with either an overnight or 2-day delivery vendor.
b. The patient should not have urinated for at least 1 h prior to specimen collection. Collect specimen in a sterile, plastic, preservative-free specimen collection cup. The patient should collect the first 15 - 20 mL of voided urine (the first part of the stream - NOT midstream).
c. If the UPP is to be added at the laboratory, store and transport urine specimens to the test site at 2 - 8°C within 4-6 days of collection.
d. If the UPP is added at the collection site, store and transport urine specimens containing a UPP to the laboratory or test site at 2 - 8°C within 4 - 6 days of collection or at 15 - 27°C within 2 days of collection.
e. Add the UPP to the urine specimen collection cup. Wear clean gloves when handling the UPP and urine specimen. The UPP must be in contact with the urine specimen for at least 2 h prior to processing. Do not freeze the urine specimen.
f. Improperly labeled specimens are rejected.
g. Improperly transported or stored specimens are rejected.

5. PROCEDURES FOR MICROSCOPIC EXAMINATIONS; CRITERIA FOR REJECTION OF INADEQUATELY PREPARED SLIDES

Not applicable for this procedure.

6. EQUIPMENT AND INSTRUMENTATION, MATERIALS, REAGENT PREPARATION, CALIBRATORS (STANDARDS), AND CONTROLS

a. Instrumentation

1. BDProbeTec ET Instrument and Instrument Plates.
2. BDProbeTec ET Lysing Heater.
3. Lysing Rack and base.
4. BDProbeTec ET Priming and Warming Heater.
5. BDProbeTec ET Pipettor and Power Supply.
6. Eppendorf Microcentrifuge, model 5415C (Brinkmann Instruments, Westbury, NY).
7. Vortex Genie mixer (Scientific Industries, Inc., Bohemia, NY).

b. Other Materials

1. BDProbeTec ET Urine Processing Kit (containing Urine Processing Pouches (UPPs)).
2. BDProbeTec ET Sample Tubes, Caps, and Pipette Tips.
3. BDProbeTec ET *Chlamydia trachomatis I Neisseria gonorrhoeae (CT/GC)* Amplified DNA Assay Endocervical Specimen Collection and DRY TRANSPORT Kit or BDProbeTec ET *Chlamydia trachomatis I Neisseria gonorrhoeae (CT/GC)* Amplified DNA Assay Collection Kit for Endocervical Specimens.
4. BDProbeTec ET *Chlamydia trachomatis I Neisseria gonorrhoeae (CT/GC)* Amplified DNA Assay Male Urethral Specimen Collection and DRY TRANSPORT Kit or BDProbeTec ET *Chlamydia trachomatis I Neisseria* gonorrhoeae *(CT/GC)* Amplified DNA Assay Collection Kit for Male Urethral Specimens.
5. Gloves, pipettes capable of delivering 1 mL, 2 mL and 4 mL.

c. Reagent Preparation

All reagents come prepackaged and are ready to use.

Each BDProbeTec ET *CT/GC* Reagent Pack contains:

- *Chlamydia trachomatis* (CT) Priming Microwells, 4 x 96.
- 4 Oligonucleotides :> 7 pmol; dNTP :>3S nmol; Detector probe ~25 pmol; with buffers and stabilIZers *Neisseria gonorrhoeae* (GC) Priming Microwells, 4 x 96.
- 4 Oligonucleotides :> 7 pmol; dNTP :> 35 nmol; Detector probe :> 25 pmol; with buffers and stabilizers *Chlamydia trachoma tis* (CT) Amplification Microwells, 4 x 96.
- Restriction enzyme :> 30 Units; DNA Polymerase 225 Units; dNTP's 280 nmol; with buffers and stabilizers. *Neisseria gonorrhoeae* (GC) Amplification Microwells, 4 x 96.
- Restriction enzyme 215 Units; DNA Polymerase 22 Units; dNTP's :>80 nmol,

with buffers and stabilizers In addition to the above reagents, the BDProbeTec ET *CT/GCJAC* Reagent Pack also contains' Amplification Control (AC) Priming Microwells, 4 x 96.

- 4 Oligonucleotides 27 pmol; dNTP :>35 nmol; Detector probe ,,25 pmol; approximately 1,000 copies per reaction of pGC10 linearized plasmid; with buffers and stabilizers.
- Amplification Control (AC) Amplification Microwells, 4 x 96.
- RestrictIon enzyme :>15 Units; DNA Polymerase 22 Units; dNTP's :>80 nmol; with buffers and stabilizers NOTE: Each microwell pouch contains one desiccant bag.
- Accessories: Priming Covers, Amplification Sealers, 40 each; Disposal Bags, 20 each.

Mix 200 mL of bleach with 800 mL of warm water. Add 7.5 g of Aleonox and mix. Prepare fresh daily

Storage and Handling Requirements: Reagents may be stored at 2 - 33°C. Unopened Reagent Packs are stable until the expiration date. Once a pouch is opened, the microwells are stable for 4 weeks if properly sealed or until the expiration date, whichever comes first. Do not freeze.

d. Standards Preparation

Calibration Standard
Not applicable for this procedure. Sample results are automatically compared against predetermined cut-off values set by the manufacturer.

e. Preparation of Quality Control Materials

Negative Control
Prepackaged and ready to use.

Positive Control
Prepackaged and ready to use.

7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

The CT/GC positive and CT/GC negative control must test as positive and negative, respectively, in order to report patient results. If controls do not perform as expected, the assay run is considered invalid and patient results will not be reported by the instrument. If the QC does not meet the expected results, repeat the entire run using a new set of controls, new microwells, and the processed specimens.

A separate Amplification Control (AC) is an option for inhibition testing and is available in the CT/GC/AC Reagent Pack. When the CT/GC/AC Reagent Pack is used, the AC must be included for each patient sample and control. The Amplification Control microwells contain approximately 1000 copies per reaction of pGC10 linearized plasmid that should be amplified in the sample matrix. The amplification control is designed to identify samples that may contain amplification inhibitors that could

prevent detection of CT or GC DNA if present.

Interpretation of Control Results

a. Control Interpretation without the AC

	CT or GC MOTA Score	Result
CT/GC Positive Control	MOTA ≥ 2000	Acceptable
CT/GC Negative Control	MOTA < 2000	Acceptable

b. Control Interpretation with the AC

	CT or GC MOTA Score	AC MOTA Score	Result
CT/GC Positive Control	MOTA ≥ 2000	MOTA ≥ 1000	Acceptable
CT/GC Negative Control	MOTA < 2000	MOTA ≥ 1000	Acceptable

NOTE: If the AC fails (MOTA < 1000), the control fails.

8. PROCEDURE OPERATING INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS

a. Instrument Preparation

1. Instrument power must be on and instruments allowed warming up prior to beginning the assay.
   a. The lysing Heater and the Priming and Warming Heater require approximately 90 min for warm-up and stabilization.
   b. The setpoint for the lysing Heater is 114°C.
   c. The setpoint for the Priming component of the Priming and Warming Heater is 72.5°C. The setpoint for the Warming component of the Priming and Warming Heater is 54°C.
   d. The BDProbeTec ET instrument is under software control and requires approximately 30 min to warm-up.
2. Heater temperatures must be checked prior to beginning the assay.
   a. Lysing Heater
      (1) Remove the plastic cover and allow temperature to equilibrate for 15 min.
      (2) The thermometer must read between 112 - 116°C.
   b. Priming and Warming Heater
      (1) The Priming Heater thermometer must read between 72 - 73°C.

(2) The Warming Heater thermometer must read between 53.5 - 54.5°C.

(3) Check the temperature displayed on the BDProbeTec ET screen. The temperature must read 47.5 - 55.0°C.

3. Pipettor

The following programs are required for performing the CT/GC assays. Program 2 is used with the CT/GC Reagent Pack. It transfers liquid from the processed samples to the CT/GC Priming Microwells. Program 3 is used with the CT/GCJAC Reagent Pack. It transfers liquid from the processed samples to the CT/GC and AC Priming Microwells. Program 5 transfers liquid from the Priming Microwells to the Amplification Microwells.

Program the pipettor as follows:

Program 1:

a. Turn the pipettor ON. The pipettor will beep once, flash "ZERO," flash software Version # and beep once more.

b. Press the blue "Prog" (Program) Key. Press the "Vol" (Volume) Key until "2" is displayed to select Program 2. Press ~ Enter."

c. To enter the programming mode, press and hold the "Prog" Key. While depressing the "prog" Key, simultaneously press the Special Function Key with a pipette tip or the end of a paper clip.

d. Press "Fill." Press the up arrow until 400 is displayed. Press "Enter."

e. Press "Disp" (Dispense). Press the up arrow key until 150 is displayed. Press "Enter"

f. Press "Disp." Press the up arrow key until 150 is displayed. Press "Enter."

g. Press "Enter" a second time to save the program and exit. You should hear a "beep" to indicate that programming is complete.

h. Verify your program by pressing the trigger to advance through each step. As you go through each step, set the speed of aspiration/dispense using the "Vol" key. At each step, the Speed indicator appears. Use the "Vol" key to adjust the speed indicator to show 2 squares for the "Fill" and "Disp" steps.

Program 2:

a. Turn the pipettor ON. The pipettor will beep once, flash "ZERO", flash Software Version # and beep once more.

b. Press the blue "Prog" (Program) Key. Press the "Vol" (Volume) Key until "3" is displayed to select Program 3.

c. Press" Enter. "

d. To enter the programming mode, press and hold the" Prog" Key. While depressing the" Prog" Key, simultaneously press the Special Function Key with a pipette tip or the end of a paper clip

e. Press" Fill" Press the up arrow until **600** is displayed. Press" Enter."

f. Press "Disp" (Dispense). Press the up arrow key until 150 is displayed.

Press "Enter."

g. Press "Disp". Press the up arrow key until 150 is displayed. Press "Enter."

h. Press "Disp". Press the up arrow key until 150 is displayed. Press "Enter."

i. Press "Enter" a second time to save the program and exit. You should hear a "beep" to indicate that programming is complete.

j. Verify your program by pressing the trigger to advance through each step. As you go through each step, set the speed of aspiration/dispense using the "Vol" key. At each step, the Speed indicator appears. Use the "Vol" key to adjust the speed indicator to show 2 squares for the "Fill" and "Disp" steps.

Program 3

a. Press the "Prog" Key. Press the "Vol" Key until "5" is displayed to select Program 5. Press "Enter."

b. To enter the programming mode, Press and hold the "Prog" Key. While depressing the "Prog" Key, simultaneously press the Special Function Key with a pipette tip or the end of a paper clip.

c. Press" Fill." Press the up arrow until **100** is displayed. Press" Enter."

d. Press "Disp." Press the up arrow key until 100 is displayed. Press "Enter."

e. Press" Mix." Press the up arrow key until **50** is displayed. Press" Enter."

f. Press "Enter" a second time to save the program and exit. You should hear a "beep" to indicate that programming is complete.

g. Verify your program by pressing the trigger to advance through each step. As you go through each step, set the speed of aspiration/dispense/mix using the "Vol" key. At each step, the Speed indicator appears. Use the "Vol" key to adjust the speed indicator to show 2 squares for the aspiration and dispense functions. Use the "Vol" key to adjust the speed for the mix so that it shows 3 squares.

Program Review

The programs should be reviewed prior to beginning the procedure. To review the programs, turn the pipettor ON. Press the blue "Prog" (Program) Key. Press the "Vol" (Volume) key until the appropriate program number (2, 3 or 5) is displayed. Press the "Enter" key. Use the pi petting trigger to advance step by step through the program.

Program 1: This program aspirates 400 µL; dispenses 150 µL into the CT microwell; dispenses 150 µL into the GC microwell. The pipettor display should read as follows:

Fill 400 µL - S II

Dispense 150 µL - S II

Dispense 150 µL - S II

Program 2: This program aspirates 600 µL; dispenses 150 µL into the CT microwell; dispenses 150 µL into the GC microwell; and dispenses 150 µL into the AC microwell. The pipettor display should read as follows:

Fill 600 µL – S 11

Dispense 150 µL - S II

Dispense 150 µL - S II

Dispense 150 µL - S II

Review Program 5 in the same way:

Program 3: This program aspirates 100 µL; dispenses 100 µL; and mixes 50 µL three times. The pipettor display should read as follows:

Fill 100 µL - S II

Dispense 100 µL - S II

Mix 50 µL - S II

Zero (flashes on and off)

b. Plate layout

The Plate Layout Report is generated from the BDProbeTec ET instrument after the assay type, specimen identification, control lot numbers, and kit lot numbers are logged into the system. The Plate Layout Report shows the physical layout of specimens and controls for each plate to be tested. The system software groups adjacent plate locations for the wells required *for* a specific assay. *For* the *CT/GC* Amplified DNA Assay, columns are assigned as follows: *CT/GC.* For the *CT/GClAC* Amplified Assay, columns are assigned as follows: *CT/GClAC.* This orientation is used for both the Priming Microwell plate and the Amplification Microwell plate.

Priming Microwells are the solid colored *microwell* strips (CT - solid green; GC - solid yellow; AC - solid black, if applicable). Amplification Microwells are the striped microwell strips (CT - striped *green;* GC - striped yellow; AC - striped black, if applicable).

c. Swab Processing

Swab specimens must be processed within 4-6 days of collection if stored at 2-27°C.

NOTE: Swabs and *CT/GC* Diluent Tubes should be at room temperature prior to use.

Processing Procedure for swabs collected with the BDProbeTec ET *CT/GC* Amplified DNA Assay Endocervical Specimen Collection and DRY TRANSPORT Kit *or* the BDProbeTec ET *CT/GC* Amplified DNA Assay Male Urethral Specimen Collection and DRY TRANSPORT Kit:

1. Label a *CT/GC* Diluent Tube for each swab specimen to be processed.
2. Remove the cap from the tube and insert the swab. Mix by swirling the swab into diluent for 5 - 10 seconds.
3. Express the specimen swab along the inside of the tube so that the liquid runs back into the bottom of the tube.

4. Remove swab carefully to avoid splashing.
5. NOTE: Droplets may cause contamination of work area.
6. Place swab back into transport tube and discard.
7. Tightly replace the cap on the *CT/GC* Diluent tube.
8. Vortex tube for 5 seconds.
9. Using the Plate Layout Report, place tube in order in the Lysing Rack.
10. Repeat steps 1-8 for additional swab specimens.
11. Lock the samples into place in the Lysing Rack. 11. Specimens are ready to be lysed.
12. NOTE: Alternatively, if a multi-tube vortexer is available, skip step g and vortex the entire Lysing Rack for 15-20 sec after step j and before Lysing.
13. NOTE: Specimens processed, but not yet lysed, may be *stored* at room temperature for up to 6 h *or* overnight at 2 - 8°C.

Processing Procedure for swabs collected with the BDProbeTec ET *CT/GC* Amplified DNA Assay Collection Kit *for* Endocervical Specimens or the BDProbeTec ET *CT/GC* Amplified DNA Assay Collection Kit for Male Urethral Specimens:

1. Vortex the *CT/GC* Diluent Tube for 5 seconds.
2. NOTE: Alternatively, if a multi-tube *vortexer* is available, perform steps 2 and 3; then vortex the entire Lysing Rack for 15-20 see and proceed to step 4.
3. Using the Plate Layout Report, place sample and control tubes in order in the Lysing Rack.
4. Lock the samples into place in the Lysing Rack.
5. Specimens are ready to be lysed.

e. Urine Processing

Urine specimens must be processed within 4-6 days of collection if stored at 2 - 8°C (UPP added at either collection or testing site) or within 2 days of collection if *stored* at 15 - 27°C (UPP added at collection site).

NOTES:

BDProbeTec ET Diluent (CTlGC) should be at *room* temperature prior to use. Urine must be in contact with the UPP *for* at least 2 h before processing.

Aliquot the needed quantity of BDProbeTec ET Diluent (CT/GC) into a clean container. To estimate the quantity needed, multiply the number of samples by 2 and add another 1 - 2 mL for pipetting ease. To avoid contamination of the Diluent - Do Note: Pour leftover Diluent back into the bottle.

1. Label an empty BDProbeTec ET Sample Tube for each urine to be processed.
2. Swirl the container to mix the urine and open carefully.

   NOTE: Open *carefully* to avoid spills or droplets which may cause contamination

of work *area.*

3. Pipette 4.0 mL of urine into the appropriately labeled tube and tightly *recap* the tube.
4. Repeat steps 2 - 3 for additional samples. Use a new pipette or pipette tip for each sample.
5. Centrifuge the tubes at 2000 x *g* for 30 min.
6. At the end of centrifugation, *carefully* remove the tubes *from* the centrifuge.
7. Uncap the first tube and carefully decant the supernatant. End the decanting motion with a gentle "flick" of the wrist to remove residual fluid from the tube.

   NOTE: This is a critical step - excess residual specimen may cause inhibition.Tubes may be individually blotted on a separate sheet of absorbent paper to enhance removal of residual urine.
8. Place the cap loosely on the tube.
9. Repeat steps 7 - 8 for each centrifuged urine specimen.
10. Pipette 2.0 mL of Diluent into each tube. Use a new pipette or pipette tip for each tube.
11. Tightly recap the sample tubes and vortex 5 sec to completely resuspend the sediment in the Diluent.
12. Samples are ready to be lysed.

    NOTE: Specimens processed, but not yet lysed, may be stored at room temperature for up to 6 h or overnight at 2 - 8°C.

f. Quality Control Preparation

   NOTE: The BDProbeTec ET (CT/GC) Controls and Diluent should be at room temperature prior to use.

   1. For each run (plate) to be tested, prepare one CT/GC Negative Control Tube and one CT/GC Positive Control Tube. If a plate contains more than one Reagent Pack lot number, controls must be tested with each lot.
   2. Remove the cap from the CT/GC Negative Control Tube. Using a new pipette tip or pipette, add 2.0 mL of Diluent
   3. Recap the tube and vortex for 5 see.
   4. Remove the cap from the CT/GC Positive Control Tube. Using a new pipette tip or pipette, add 2.0 mL of Diluent.
   5. Recap the tube and vortex for 5 see.
   6. Controls are ready to be lysed.

g. Lysing the Samples and Controls

   1. Insert the lysing Rack into the Lysing Heater.
   2. Heat the samples for 30 min.

3. After 30 min, remove the Lysing Rack from the Lysing Heater and allow cooling at room temperature for at least 15 min.

   NOTE: After lysing samples:

   a. They may be stored at 18 - 30°C for up to 6 h and may be tested without re-lysing.

   b. They may be stored up to 5 days at 2 - 8°C. Samples must be vortexed and re-lysed prior to testing.

   c. They may be stored up to 98 days at s -20°C. Samples must be thawed at room temperature, vortexed and re lysed prior to testing. Lysed samples may be frozen and thawed twice.

h. Testing Procedure for the CT/GC Reagent Pack

   NOTE: The Priming and Amplification Microwells should be at room temperature prior to use.

   1. For specimens collected with the BDProbeTec ET CT/GC Amplified DNA Assay Endocervical Specimen Collection and DRY TRANSPORT Kit or the BDProbeTec ET CT/GC Amplified DNA Assay Male Urethral Specimen Collection and DRY TRANSPORT Kit, remove and discard the caps from the lysed and cooled samples and controls.
   2. For swabs collected with the BDProbeTec ET CT/GC Amplified DNA Assay Collection Kit for Endocervical Specimens or the BDProbeTec ET CT/GC Amplified DNA Assay Collection Kit for Male Urethral Specimens, do the following:

      a. Uncap the tube and gently press the swab against the side of the tube to remove excess fluid.

      b. Pull the cap/swab out of the tube. Do not press against the wall of tube to avoid splattering droplets which may cause cross-contamination.

      c. Discard the cap/swab.

   3. Change gloves before proceeding to avoid contamination.
   4. Using the Plate Layout Report, prepare the priming plate. The Priming Microwells must be placed in a plate in the following order: CT (solid green microwells) then GC (solid yellow microwells). Repeat until plate is configured like the Plate Layout Report.
   5. Re-seal the Microwell pouches as follows.

      a. Place pouch on flat surface. Hold the open end flat with one hand.

      b. While applying pressure, slide finger across outside of seal moving from one edge of pouch to other.

      c. Inspect to ensure pouch is sealed.

   6. Select Program 2 on the BDProbeTec ET Pipettor.
   7. Pick up pipette tips. Expand the pipettor by pulling the spacing knob all the way out.

NOTE: Make sure tips are fitted securely on the pipettor to prevent leakage.

8. Aspirate 400 ~L from the 1st column of samples.
9. Gently collapse the pipettor, touch pipette tips to sides of wells and dispense 150 ~L into each of the 2 corresponding columns of Priming Microwells (1 A-H; 2 A-H).

   NOTE: Do not collapse pipettor over samples or microwells as this may cause contamination. Abrupt movements may cause droplets or aerosols.

   NOTE: It is important to dispense liquids against the inside wall of microwells to assure accuracy and precision and to avoid cross-contamination.
10. Discard tips. Depress the pipetting trigger to reset the pipettor.

    NOTE: Discard tips carefully to avoid droplets or aerosols which may contaminate the work area.
11. Pick up new tips and aspirate 400 µL from the 2nd column of samples.
12. Gently collapse the pipettor, touch pipette tips to sides of wells and dispense 150 µL into each of the 2 corresponding columns of Priming Microwells (3 A-H; 4 A-H).
13. Discard tips.
14. Continue transferring the remaining samples for the run.
15. Cover the Priming Microwell plate with the Priming Cover and let plate incubate at room temperature for at least 20 min. (May incubate up to 6 h.)

    NOTE: Recap the processed samples with new caps to retain the sample tubes.
16. At the end of the priming incubation prepare the amplification plate. Configure the Amplification Microwells in a plate to match the Plate Layout Report (same as the priming plate). Reseal the microwell pouches as described in step #4.
17. Remove the cover from the Priming Microwell plate and place the plate in the Priming Heater. IMMEDIATELY place the Amplification Microwell plate in the Warming Heater to pre-warm.
18. Set timer for 10 min. (NOTE: This step is time critical.).
19. At the end of the 10 min (+/- 1 min) incubation, select Program 5 on the pipettor.
20. Pick up tips and transfer 100 µL from column 1 of the Priming Microwell plate to column 1 of the Amplification Microwell plate. Allow pipette tips to touch sides of wells and dispense the liquid. After dispensing, allow pipettor to automatically mix the liquid in the wells. Carefully lift the pipettor away from the plate. Avoid touching other wells.
21. Discard tips. Pick up new tips and continue to transfer the reaction mixture from the Priming Microwells to the Amplification Microwells, column by column, using new tips for each column.
22. When the last column has been transferred, remove the backing from an Amplification Sealer (remove one half of the sealer backing if 6 or fewer

columns are occupied by microwells; remove the entire backing if 7 or more columns are occupied by microwells). Hold the sealer by the edges and center over the plate. Use the guides on the Warming Heater to assist you in centering the sealer. The sealer will extend over the microwells on both sides of the plate. Press downward on sealer to ensure that all microwells are completely sealed.

23. At the BDProbeTec ET user interface, move the carrier out and open the doors. IMMEDIATELY (within 30 see) transfer the sealed Amplification Microwell plate to the BDProbeTec ET Instrument and initiate the run. (Refer to the BDProbeTec ET System User's Manual for detailed instructions.).
24. After initiating the run, complete the following portion of the clean-up procedure:
    a. Seal the Priming Microwells with an Amplification Sealer and remove plate from the Priming and Warming Heater.

       WARNING: Temperature is in excess of 70°C, Use the heat resistant glove to remove the plate.

    b. Allow the plate to cool on the bench for 5 min.
    c. Remove the sealed Priming Microwells from the plate by holding the top and bottom of the sealer and lifting the wells straight up as a unit. Place the Microwells into a Disposal Bag and seal.
    d. Clean the metal plate:

       Rinse the plate with the 1 % (v/v) sodium hypochlorite with Alconox solution.

       Rinse the plate with water.

       Wrap plate in a clean towel and allow to completely dry prior to re-use.
25. When the run is complete, a printout of the test results will be generated.
26. Move the plate carrier out of the stage, open the door, and remove the plate. Close the door and return the plate stage to the inside of the instrument.
27. Remove the sealed Amplification Microwells from the plate. CAUTION: Do Not Remove Sealing Material from Microwells. The sealed microwells may be easily removed as a unit by holding the sealer at the top and bottom and lifting straight up and out of the plate. Place the sealed microwells into the Disposal Bag. Seal the bag.
28. Clean the metal plate:

    Rinse the plate with 1 % (v/v) sodium hypochlorite with Alconox solution. Rinse the plate with water.

    Wrap plate in a clean towel and allow to completely dry prior to re-use.
29. After the last run of the day, perform the following clean-up procedures:
    a. Saturate paper towels or gauze pads with the 1 % (v/v) sodium hypochlorite with Alconox solution and apply to countertops and the exterior surfaces of the Lysing Heater, Priming and Warming Heater, and the BDProbeTec ET Instrument. Allow the solution to remain on surfaces

for 2 - 3 min. Saturate paper towels or gauze pads with water and remove cleaning solution. Change towels or gauze frequently when applying cleaning solution and when rinsing with water. Dampen paper towels or gauze pads with 1 % (v/v) sodium hypochlorite with Aleonox and wipe the Pipettor handle (ONLY THE HANDLE). After 2 - 3 min wipe the handle with paper towels or gauze pads dampened with water.

b. Immerse the Lysing Rack, Lysing Rack base, Lysing Rack cover and plates in 1 % sodium hypochlorite with Aleonox for 1 - 2 min. Rinse thoroughly with water and allow to air dry.

c. Recharge the Pipettor.

d. Dispose of sealed Disposal Bag and biohazard bag according to established procedures for disposal of contaminated biological waste material.

i. Testing Procedure for the CT/GC/AC Reagent Pack

NOTE: The Priming and Amplification Microwells should be at room temperature prior to use.

1. For specimens collected with the BDProbeTec ET CT/GC Amplified DNA Assay Endocervical Specimen Collection and DRY TRANSPORT Kit or the BDProbeTec ET CT/GC Amplified DNA Assay Male Urethral Specimen Collection and DRY TRANSPORT Kit, remove and discard the caps from the lysed and cooled samples and controls.
2. For swabs collected with the BDProbeTec ET CT/GC Amplified DNA Assay Collection Kit for Endocervical Specimens or the BDProbeTec ET *CT/GC* Amplified DNA Assay Collection Kit for Male Urethral Specimens, do the following:
   a. Uncap the tube and gently press the swab against the side of the tube to remove excess fluid.
   b. Pull the cap/swab out of the tube. Do not press against the wall of tube to avoid splattering droplets which **may cause** cross-contamination.
   c. Discard the cap/swab.
3. Change gloves before proceeding to avoid contamination.
4. Using the Plate Layout Report, prepare the priming plate. The Priming Microwells must be placed in a plate in the following order: *CT* (solid green microwells), GC (solid yellow microwells), and AC (solid black microwells). Repeat until plate is configured like the Plate Layout Report.
5. Re-seal the Microwell pouches as follows.
   a. Place pouch on flat surface. Hold the open end flat with one hand.
   b. While applying pressure, slide finger across outside of seal moving from one edge of pouch to other.
   c. Inspect to ensure pouch is sealed.
6. Select Program 3 on the BDProbeTec ET Pipettor.
7. Pick up pipette tips. Expand the pipettor by pulling the spacing knob all the way

out.

NOTE: Make sure tips are fitted securely on the pipettor to prevent leakage.

8. Aspirate 600 ~L from 1st column of samples.
9. Gently collapse the pipettor, touch pipette tips to sides of wells and dispense 150 ~L into each of the 3 corresponding columns of Priming Microwells (1 A-H; 2 A-H; 3 A-H).

   NOTE: Do not collapse pipettor over samples or microwells as this may cause contamination. Abrupt movements may cause droplets or aerosols.

   NOTE: It is important to dispense liquids against the inside wall of microwells to assure accuracy and precision and to avoid cross-contamination.
10. Discard tips. Depress the pipetting trigger to reset the pipettor.

    NOTE: Discard tips carefully to avoid droplets or aerosols which may contaminate the work area.
11. Pick up new tips and aspirate 600 ~L from the 2nd column of samples.
12. Gently collapse the pipettor, touch pipette tips to sides of wells and dispense 150 ~L into each of the 3 corresponding columns of Priming Microwells (4 A-H; 5 A-H; 6 A-H).
13. Discard tips.
14. Continue transferring the remaining samples for the run.
15. Cover the Priming Microwell plate with the Priming cover and let plate incubate at room temperature for at least 20 min. (May incubate up to 6 h.)

    NOTE: Recap the processed samples with new caps to retain the sample tubes.
16. At the end of the priming incubation prepare the amplification plate. Configure the Amplification Microwells in a plate to match the Plate Layout Report (same as the priming plate). Reseal the Microwell pouches as described in step #4.
17. Remove the cover from the Priming Microwell plate and place the plate in the Priming Heater. IMMEDIATELY
    Place the Amplification Microwell plate in the Warming Heater to pre-warm.
18. Set timer for **10** min. (NOTE: This step is time critical.).
19. At the end of the 10 min (+/- 1 min) incubation, select Program 5 on the pipettor.
20. Pick up tips and transfer 100 ~L from column 1 of the Priming Microwell plate to column 1 of the Amplification Microwell plate. Allow pipette tips to touch sides of wells and dispense the liquid. After dispensing, allow pipettor to automatically mix the liquid in the wells. Carefully lift the pipettor away from the plate. Avoid touching other wells.
21. Discard tips. Pick up new tips and continue to transfer the reaction mixture from the Priming Microwelis to the Amplification Microwells, column by column, using new tips for each column.
22. When the last column has been transferred, remove the backing from an

Amplification sealer (remove one half of the sealer backing if 6 or fewer columns are occupied by microwells; remove the entire backing if 7 or more columns are occupied by microwells). Hold the sealer by the edges and center over the plate. Use the guides on the Warming Heater to assist you in centering the sealer. The sealer will extend over the microwells on both sides of the plate. Press downward on sealer to ensure that all microwells are completely sealed.

23. At the BDProbeTec ET user interface, move the carrier out and open the doors. IMMEDIATELY (within 30 sec) transfer the sealed Amplification Microwell plate to the BDProbeTec ET Instrument and initiate the run.
24. After initiating the run, complete the following portion of the clean-up procedure:
    a. Seal the Priming Microwells with an Amplification Sealer and remove plate from the Priming and Warming Heater.

       WARNING: Temperature is in excess of 70°C. - Use the heat resistant glove to remove the plate.

    b. Allow the plate to cool on the bench for 5 min.
    c. Remove the sealed Priming Microwells from the plate by holding the top and bottom of the sealer at and lifting the wells straight up as a unit. Place the Microwells into a Disposal Bag and seal.
    d. Clean the metal plate:

       Rinse the plate with the 1 % (v/v) sodium hypochlorite with Alconox solution.

       Rinse the plate with water.

       Wrap plate in a clean towel and allow to completely dry prior to re-use.
25. When the run is complete, a printout of the test results will be generated.
26. Move the plate carrier out of the stage, open the door, and remove the plate. Close the door and return the plate stage to the inside of the instrument.
27. Remove the sealed Amplification Microwells from the plate. CAUTION: Do Not Remove Sealing Material from Microwells. The sealed microwells may be easily removed as a unit by holding the top and bottom of the sealer and lifting straight up and out of the plate. Place the sealed microwells into the Disposal Bag. Seal the bag.
28. Clean the metal plate:

    Rinse the plate with 1 % (v/v) sodium hypochlorite with Alconox solution.

    Rinse the plate with water.

    Wrap plate in a clean towel and allow to completely dry prior to re-use.
29. After the last run of the day, perform the following clean-up procedures:
    a. Saturate paper towels or gauze pads with the 1 % (v/v) sodium hypochlorite with Alconox solution and apply to countertops and the exterior surfaces of the Lysing Heater, Priming and Warming Heater, and the BDProbeTec ET Instrument. Allow the solution to remain on surfaces for 2 - 3 min. Saturate

paper towels or gauze pads with water and remove cleaning solution. Change towels or gauze frequently when applying cleaning solution and when rinsing with water. Dampen paper towels or gauze pads with 1 % (v/v) sodium hypochlorite with Alconox and wipe the Pipettor handle (ONLY THE HANDLE). After 2 - 3 min wipe the handle with paper towels or gauze pads dampened with water.

b. Immerse the Lysing Rack, Lysing Rack base, Lysing Rack cover and plates in 1 % (v/v) sodium hypochlorite with Alconox for 1 - 2 min. Rinse thoroughly with water and allow to air dry.

c. Recharge the Pipettor.

d. Dispose of sealed Disposal Bag and biohazard bag according to established procedures for disposal of contaminated biological waste material.

9. REPORTABLE RANGE OF RESULTS

Reportable results are expressed as positive or negative.

10. QUALITY CONTROL (QC) PROCEDURES

The BDProbeTec ET *Chlamydia trachomatis / Neisseria gonorrhoeae* positive and negative control set is provided separately. One positive and one negative control must be included in each assay run and for each new reagent kit lot number. Controls may be randomly positioned. The CT/GC positive control will monitor for substantial reagent failure only. The CT/GC negative control monitors for reagent and/or environmental contamination.

The positive control has both cloned CT and GC target regions that are not necessarily representative of organism target DNA detected by the assay nor do they represent specimen matrices (urine and epithelial cell suspensions) indicated for use with the BDProbeTec ET System. These controls may be used for internal quality control or users may develop their own internal quality control material, as described by NCCIS C24-A2.[14] Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations. Refer to NCCLS C24-A2 for additional guidance on appropriate internal quality control testing practices. The positive control contains 750 copies per reaction of pCT16 linearized plasmid and 250 copies per reaction of pGC10 linearized plasmid. Both organisms have multiple copies of the target The BDProbeTec ET amplification reaction volume is 100 µl of rehydrated control.

Because the CT/GC positive control is used for both CT and GC testing, correct positioning of the microwell strips is important for proper result reporting. Refer to Section h of the "Test Procedure" for correct microwell strip positioning.

a. Specimen Processing Controls

Specimen processing controls may be tested in accordance with the requirements of appropriate accrediting organizations. A positive control should

test the entire assay system. For this purpose, known positive specimens can serve as controls by being processed and tested in conjunction with unknown specimens. Specimens used as processing controls must be stored, processed, and tested according to the package insert. Specimen processing controls which simulate urine processing can also be prepared as described below.

Chlamydia trachomatis:

If a known positive specimen is not available, another approach is to assay a stock culture of *C. trachomatis* LGV2 (ATCC VR·902B) prepared as described below:

1. Thaw a vial of C. *trachomatis* LGV2 cells ATCC VR-902B.
2. Prepare 10-fold serial dilutions to a 10^5 dilution (at least 5 mL final volume) in phosphate buffered saline (PBS).
3. Place 4 mL of 10^5 dilution in a BDProbeTec ET sample tube.
4. Process as a urine sample starting at Section E, step 5 of the "Test Procedure."
5. After processing, lyse sample as described in Section G of the "Test Procedure." 6. Continue testing as described in Section H of the "Test Procedure."

Neisseria gonorrhoeae

If a known positive specimen is not available, another approach is to assay a stock culture of *N. gonorrhoeae* (available from the ATCC, 19424) prepared as described below:

1. Thaw a vial of *N. gonorrhoeae* stock culture and immediately inoculate a chocolate agar plate.
2. Incubate at 37°C in 3 - 5% CO_2 for 24 - 48 h.
3. Resuspend colonies from the chocolate agar plate with phosphate buffered saline (PBS).
4. Dilute cells in PBS to a 1.0 McFarland turbidity standard (approximately 3 X 10^8 cells/mL).
5. Prepare 10-fold serial dilutions to a 10^5 dilution of the McFarland (at least 5 mL final volume) in PBS.
6. Place 4 mL of the 10^s dilution in a BDProbeTec ET sample tube.
7. Process as a urine sample starting at Section E, step 5 of the "Test Procedure."
8. After processing, lyse sample as described in Section G of the "Test Procedure."
9. Continue testing as described in Section H of the "Test Procedure."

b. Monitoring for the Presence of DNA Contamination

At least monthly, the following test procedure should be performed to monitor the work area and equipment surfaces for the presence of DNA contamination.

Environmental monitoring is essential to detect contamination prior to the development of a problem.

For each area to be tested, use a clean collection swab from either of the BDProbeTec ET Endocervical specimen collection and transport systems and a *CT/GC* Diluent tube. [Alternatively, a sample tube containing 2 mL of Diluent (CT/GC), may be used.].

Dip the swab into the *CT/GC* Diluent and wipe the first area* using a broad sweeping motion.

Express the swab in the *CT/GC* Diluent tube. Recap the tube and vortex for 5 seconds.

Repeat for each desired area.

After all swabs have been collected, expressed in diluent and vortexed, the tubes are ready to be lysed (Section G) and assayed (Section H) according to the "Test Procedure."

Recommended areas to test include: surface of the Lysing Heater, Lysing Rack, Priming and Warming Heater, black microwell trays, pipettor handle, instrument touch keys, instrument keyboard, instrument door release (teal key) centrifuge drum, and work bench(es) including sample processing areas.

If an area gives a positive result, clean the area with fresh 1 % (v/v) sodium hypochlorite with Alconox. Make sure the entire area is wetted with the solution and allowed to remain on the surface for at least two minutes or until dry. If necessary, remove excess cleaning solution with a clean towel. Wipe the area with a clean towel saturated with water and allow the surface to dry. Retest the area. Repeat until negative results are obtained. If the contamination does not resolve, contact Technical Services for additional information.

c, Interpretation of Test Results

The BDProbeTec ET *Chlamydia trachomatis* and *Neisseria gonorrhoeae* Amplified DNA Assay uses fluorescent energy transfer as the detection method to test for the presence of C. *trachomatis* and *N. gonorrhoeae* in clinical specimens. All calculations are performed automatically by the instrument software.

The presence or absence of C. *trachomatis* and *N. gonorrhoeae* is determined by relating the BDProbeTec ET MOTA scores for the specimen to pre-determined cutoff values. The MOTA score is a metric used to assess the magnitude of signal generated as a result of the reaction. The magnitude of the MOTA score is not indicative of the level of organism **in the specimen.**

If assay controls are not as expected, patient results should not be reported. See QC section for expected control values. Reported results are determined as follows.

For the CT/GC Reagent Pack

C. *trachomatis* and *N. gonorrhoeae* Result Interpretation without the AC

CT or GC MOTA Score	Report	Interpretation	Result

≥ 10,000	*C. trachomatis* plasmid DNA and/or *N. gonorrhoeae* DNA detected by SDA	Positive for *C. trachomatis* and/or *N. gonorrhoeae*. *C. trachomatis* and/or *N. gonorrhoeae* organism viability and/or infectivity cannot be inferred since target DNA may persist in the absence of viable organisms.	Positive
2,000-9,999	*C. trachomatis* plasmid DNA and/or *N. gonorrhoeae* DNA detected by SDA	*C. trachomatis* and/or *N. gonorrhoeae* likely. Repeat testing may be useful for verifying presence of *C. trachomatis* and/or *N. gonorrhoeae*	Low Positive
< 2,000	*C. trachomatis* plasmid DNA and/or *N. gonorrhoeae* DNA NOT detected by SDA	Presumed negative for *C. trachomatis* and/or *N. gonorrhoeae*. A negative result does not preclude *C. trachomatis* and/or *N. gonorrhoeae* infection because results are dependent on adequate specimen collection, absence of inhibitors, and sufficient DNA to be detected	Negative

For the CT/GC/AC Reagent Pack

C. *trachomatis* and *N. gonorrhoeae* Result Interpretation with the AC

CT or GC MOTA Score	AC MOTA Score	Report	Interpretation	Result
≥ 10,000	Any	*C. trachomatis* plasmid DNA and/or *N. gonorrhoeae* DNA detected by SDA	Positive for *C. trachomatis* and/or *N. gonorrhoeae*. *C. trachomatis* and/or *N. gonorrhoeae* organism viability and/or infectivity cannot be inferred since target DNA may persist in the absence of viable organisms.	Positive
2,000-9,999	Any	*C. trachomatis* plasmid DNA and/or *N. gonorrhoeae* DNA detected by SDA	*C. trachomatis* and/or *N. gonorrhoeae* likely. Repeat testing may be useful for verifying presence of *C. trachomatis* and/or *N. gonorrhoeae*	Low Positive
< 2000	≥ 1000	*C. trachomatis* plasmid DNA and/or *N. gonorrhoeae* DNA NOT detected by SDA	Presumed negative for *C. trachomatis* and/or *N. gonorrhoeae*. A negative result does not preclude *C. trachomatis* and/or *N. gonorrhoeae* infection because results are dependent on adequate specimen collection, absence of inhibitors, and sufficient DNA to be detected	Negative
<2000	<1000	Amplification Control inhibited. Repeat test.	Repeatedly inhabited specimen. *C. trachomatis* and/or *N. gonorrhoeae*, if present, would not be detectable using SDA. Submit another specimen for testing.	Indeterminate

12. LIMITATIONS OF THE PROCEDURE

a. This method has been tested only with endocervical swabs, male urethral swabs, and male and female urine specimens. Performance with other specimen types has not been assessed.

b. Optimal performance of the test requires adequate specimen collection and handling.

c. Endocervical specimen adequacy can only be assessed by microscopic visualization of columnar epithelial cells in the specimens.

d. Collection and testing of urine specimens with the BDProbeTec ET *Chlamydia*

trachomatis / *Neisseria gonorrhoeae* Amplified DNA Assay is not intended to replace cervical exam and endocervical sampling for diagnosis of urogenital infection. Cervicitis, urethritis, urinary tract infections and vaginal infections may result from other causes or concurrent infections may occur.

e. The BDProbeTec ET *Chlamydia trachomatis* / *Neisseria gonorrhoeae* Amplified DNA Assay for male and female urine testing should be performed on first catch random urine specimens (defined as the first 15 - 20 mL of the urine stream). During the clinical evaluation, testing urine volumes up to 60 mL was included in the performance estimates. Dilutional effects of larger urine volumes may result in reduced assay sensitivity. The effects of other variables such as mid-stream collection have not been determined. Performance has not been established when the UPP is added to the collection cup prior to collection.

f. The effects of other potential variables such as vaginal discharge, use of tampons, douching, and specimen collection variables have not been determined.

g. A negative test result does not exclude the possibility of infection because test results may be affected by improper specimen collection, technical error, specimen mix-up, concurrent antibiotic therapy, or the number of organisms in the specimen which may be below the sensitivity of the test.

h. As with many diagnostic tests, results from the BDProbeTec ET *Chlamydia trachomatis* / *Neisseria gonorrhoeae* Amplified DNA Assay should be interpreted in conjunction with other laboratory and clinical data available to the physician.

i. The BDProbeTec ET *Chlamydia trachomatis* / *Neisseria gonorrhoeae* Amplified DNA Assay does not detect plasmid free variants of *C. trachomatis*.

j. The BDProbeTec ET *Chlamydia trachomatis* / *Neisseria gonorrhoeae* Amplified DNA Assay should not be used for the evaluation of suspected sexual abuse or for other medico-legal indications. Additional testing is recommended in any circumstance when false positive or false negative results could lead to adverse medical, social, or psychological consequences.

k. The BDProbeTec ET system cannot be used to assess therapeutic success or failure since nucleic acids from *Chlamydia trachomatis* and *Neisseria gonorrhoeae* may persist following antimicrobial therapy.

l. The BDProbeTec ET *Chlamydia trachomatis* / *Neisseria gonorrhoeae* Amplified DNA Assay provides qualitative results.

m. No correlation can be drawn between the magnitude of MOTA score and the number of cells in an infected sample.

n. The predictive value of an assay depends on the prevalence of the disease in any particular population.

o. Because the CT/GC positive control is used for both CT and GC testing, correct positioning of the microwell strips is important for final results reporting. Refer to Section H of the "Test Procedure" for correct microwell strip positioning.

p. Use of the BDProbeTec ET *Chlamydia trachomatis* / *Neisseria gonorrhoeae* Amplified DNA Assay is limited to personnel who have been trained in the assay procedure and the BDProbeTec ET system.

q. In laboratory studies, blood> 5% (vlv) was shown to cause indeterminate (inhibitory) results in both urine and swab specimens (with AC) and false negative results in urine specimens (with and without AC). Blood> 5% (vlv) may cause false negative results in swab specimens (with and without AC). Specimens with moderate to gross blood may interfere with BDProbeTec ET CT/GC Assay results.

r. The presence of highly pigmented substances in urine, such as bilirubin (10 mg/mL) and Phenazopyridine (10 mg/mL), may cause indeterminate or false negative results.

s. Leukocytes in excess of 250,000 cells/mL (swab specimens) may cause indeterminate or false negative results.

t. The BDProbeTec ET *Chlamydia trachomatis* / *Neisseria gonorrhoeae* Amplified DNA Assays may cross-react with *N. cinerea* and *N. lactamica*.

u. The reproducibility of the BDProbeTec ET CT/GC Assay was established using seeded swab specimens and seeded buffer to simulate urine specimens. These specimens were inoculated with both *C. trachomatis* and *N. gonorrhoeae*. Reproducibility when testing urine samples and samples with *C. trachomatis* only and *N. gonorrhoeae* only has not been determined.

v. Performance characteristics for detecting *N. gonorrhoeae* in males are based on testing patients with infection rates of 0-43%; the male populations sampled were primarily from STD clinics where the prevalence of GC is higher than in other clinical settings. In males, 16 gonococcal infections were identified in the low prevalence setting (0-8% prevalence). Likewise, the majority of females in the study with GC infections were from STD clinics. In females, only six gonococcal infections were identified in the low prevalence setting (1.2% prevalence). Positive results in low prevalence populations should be interpreted carefully in conjunction with clinical signs and symptoms, patient risk profile, and other findings with the understanding that the likelihood of a false positive may be higher than a true positive.

w. Testing urine specimens from female patients as the sole test for identifying chlamydial or gonococcal infections may miss infected individuals (17/100 or 17% of females with CT-positive cultures and 11/80 or 13.8% of females with GC-positive cultures had negative results when urine only was tested) with the BDProbeTec ET CT/GC Assay.

x. Because the AC uses GC target, the efficacy of the AC for detecting inhibition is reduced in GC infected samples.

13. REFERENCE RANGES (NORMAL VALUES)

Not applicable to this procedure.

14. CRITICAL CALL RESULTS (PANIC VALUES)

Not applicable to this procedure.

15. SPECIMEN STORAGE AND HANDLING DURING TESTING

Keep the specimens at 20–25°C during preparation and testing. Otherwise, store the serum at ≤–20°C.

16. ALTERNATIVE METHODS FOR PERFORMING TEST OR STORING SPECIMENS IF TEST SYSTEM FAILS

In case of system failure, another FDA cleared amplified technology will be used to test the specimens.

17. TEST RESULT REPORTING SYSTEM; PROTOCOL FOR REPORTING CRITICAL CALLS (IF APPLICABLE)

Not applicable for this procedure.

18. TRANSFER OR REFERRAL OF SPECIMENS; PROCEDURES FOR SPECIMEN ACCOUNTABILITY AND TRACKING

For the NHANES 2005–2006 study, residual urine samples are stored at ≤–20°C for 10 years after analysis and then discarded.

19. SUMMARY STATISTICS AND QC GRAPHS

Qualitative assays are qualitative assays with a positive, negative or borderline/indeterminate result. The absorbance or reactivity values of specimens are compared with a cutoff value that is a ratio of the negative control mean and the positive control mean. Since the controls are read as cutoff values, plots of these values are not generated for quality control purposes.

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